Author's response to reviews

Title: Reduced levels of circulating progenitor cells in juvenile idiopathic arthritis are counteracted by anti TNF-alpha therapy

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Author's response to reviews:

REVIEWER NUMBER 1

Several aspects would require further clarification:

1- Methods
   a. What was the biological sample that was tested: peripheral blood, RCB lysed PB, PBMC?

   Our reply. We have now specified that we used fresh whole blood, analyzed after RBC lysis.

   b. Enumeration of CD34 according to the ISHAGE guidelines is required for the determination of viable CD34+ absolute count. Have the authors tested CD34 viability?

   Our reply. We did not apply the ISHAGE protocol, but a FACS protocol optimized over 10 years in our laboratory, as referenced. CD34+ cell viability was not tested.

   c. How was the absolute number of CD34 quantified? Have the authors included beads to determine this?

   Our reply. The absolute levels or progenitor cell phenotypes have now been calculated by multiplying relative levels per WBC counts, and included in the revised manuscript.

2- Table 1
   a. No reference to the demographic characteristics of controls is provided (gender, co-morbidities, CRP)
Our reply. These data were provided in the text.

b. What is the meaning of the acronyms NPX and PDN that are used in the table?

Our reply. Acronyms in table 1 have been expanded.

3- Table 2 – Fig 1
a. It is unclear what is the physiological meaning of the categorization of the CPC that is provided in this table
b. No need to duplicate the information of Table 2 in Fig 1

Our reply. In the revised manuscript, we have described the different physiological meaning of the various progenitor cell phenotypes. Table 2 has been deleted.

4- Fig 2
a- It is unclear what the authors call visual activity score. Is this patient global (visual analogue scale from 0-10)? It would be relevant to include composite measures of disease activity (DAS28, CDAI, SDAI)?

b- Since the individuals included in this study have ‘VAS 7-10’ (except fro 3 individuals) the correlation with disease activity can’t be supported

Our reply. VAS was the physician’s visual analogue score. DAS28 and other composite measures of disease activity were not used at the time the study was performed. Relevance of this correlation has been tapered, as being driven by 3 patients with low activity.

5- Fig 3
a. Does the ‘normalization’ of the CPC values correlate with response to anti-TNF? Were all the patients in remission 6 mo following anti-TNF treatment? The 3 patients in whom data is presented at 1 year – did they have sustained remission?

Our reply. Yes, restoration of CPC levels was paralleled by clinical response to anti-TNF, as evidenced by reduction of CRP, VES and number of active joints. We have included these data in the revised manuscript.

6- Fig 4
a. There is lack of lack of histology and quantitative analysis. Unclear what do the authors call CD34+ rounded cells. The representative images provided do not allow excluding that the differences observed are not related to variations in vascularization.

Our reply. Putative EPCs were not detected in the control synovial tissue, thus making quantitative comparison impossible and useless. It is in fact possible that differences observed in EPC are own to differences in vascularisation, which a typical pathologic feature of JIA synovitis. “Rounded” means that cells are not
typically elongated as endothelial cells and are isolated.

7- Based on the descriptive nature of this study and the presumptive normalization of the CPC counts with anti-TNF treatment it is speculative to suggest that a reduction in CPC ‘entails an excess cardiovascular risk in JIA, which can impact survival and morbidity later in life’

Our reply. According to this reviewer comment, in the revised manuscript, we have tapered this conclusion.

REVIEWER NUMBER 2
Referee's comments to the author(s)
In the introduction, the authors state that at least in some rheumatic conditions, the EPC rise driven by acute inflammation during the early stages of the disease followed by depletion in the chronic phase. In the described group of JIA patients disease duration ranged from 0.2 to 17.4 years. Any relationship between EPC numbers and disease duration?

Our reply. We did not find any relationship between progenitor cells and disease duration. This has been added to the result section of the revised manuscript.

Did patients treated with to TNF–inhibiting agents show a good clinical response?

Our reply. Yes, restoration of CPC levels was paralleled by clinical response to anti-TNF, as evidenced by reduction of CRP, VES and number of active joints. We have included these data in the revised manuscript.

The synovial tissue samples were obtained from 3 patients who underwent arthroscopic synovectomy. Did those patients had monoarthritis? Was the synovial pathology in these patients consistent with the diagnosis of JIA? Were PVNS or similar conditions ruled out? What was the underlying diagnosis of the patient with “non-inflammatory disease”?

Our reply. Synovial tissue was obtained at time of arthroscopic synovectomy in 3 patients with oligoarticular-onset JIA. The diagnosis was consistent with JIA and not PVNS. The control subject with non-inflammatory disease who underwent synovectomy was affected by hypermobile joint.

Minor problems:
The list of references has 26 articles. In the manuscript there are references to ref 27-34

Our reply. Referenced have been reformatted.